

Investigation of estrogen activity in the raw and treated waters of riverbank infiltration using a yeast estrogen screen and chemical analysis

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ABSTRACT

Exposure to various endocrine disrupting chemicals (EDCs) can lead to adverse effects on reproductive physiology and behavior in both animals and humans. An adequate strategy for the prevention of environmental contamination and eliminating the effects of them must be established. Chemicals with estrogenic activity were selected, and the effectiveness of their removal during the purification processes in two drinking water treatment plants (DWTPs) using riverbank infiltrated water was determined. Thirty-five water samples in two sampling campaigns throughout different seasons were collected and screened with a yeast estrogen test; furthermore, bisphenol A (BPA), 17 β -estradiol (E2) and ethinyl-estradiol (EE2) content were measured using HPLC-MS. Our results confirm that estrogenic compounds are present in sewage effluents and raw surface river water of DWTPs. Very low estrogen activity and pg/L concentrations of BPA and E2 were detected during drinking water processing and occasionally in drinking water. Based on this study, applied riverbank infiltration and water treatment procedures do not seem to be suitable for the total removal of estrogenic chemicals. Local contamination could play an important role in increasing the BPA content of the drinking water at the consumer endpoint.

Key words | 17 β -estradiol, bisphenol A, drinking water, ethinyl-estradiol, riverbank infiltration, yeast estrogen test

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INTRODUCTION

Endocrine disrupting compounds (EDCs) can be classified into different groups. These groups include synthetic and natural hormones, drugs with hormone-like side effects, phyto- and mycoestrogens, industrial and household chemicals, products or byproducts of industrial and household processes, pesticides and their metabolites. Certain heavy metals, such as cadmium and lead, are also known to affect the endocrine system (Diamanti-Kandarakis *et al.* 2009; Hong 2012; Zlatnik 2016). Exposure to various EDCs can lead to adverse effects on the reproductive system in both animals and humans (Trasande *et al.* 2016; Vandenberg

et al. 2016; Zlatnik 2016). Environmental samples contain a mixture of low potency disruptors, such as surfactants in $\mu\text{g/L}$ and synthetic or natural estrogens in ng/L concentration levels (Céspedes *et al.* 2004). Different mechanisms and signal transduction pathways underlying the effects of EDCs with lower hormonal activity are poorly understood. An adequate strategy for the prevention of environmental contamination and to eliminate the effects of them must be established (Patisaul & Adewale 2009). Understanding the complexity of human exposure to chemicals that have an effect upon the functions of the hormone system is

compromised by the overwhelming number of synthetic chemicals and chemicals of natural origin in use and the technical limitations of their detection. Thus, research studies are focused on a few chemicals as proxies for the total exposure, such as 17 β -estradiol (E2), ethinyl-estradiol (EE2), bisphenol A (BPA), nonylphenol and phthalic acid esters, for which more monitoring data are available (Kuch & Ballschmiter 2001; Carvalho *et al.* 2015; Avar *et al.* 2016a, 2016b; Praveena *et al.* 2016); however, it is questionable whether these compounds adequately represent the total exposure (Wagner & Oehlmann 2011). Biological assays are suitable for first screening and helping to target sites with problems without any previous knowledge of what chemicals may be present (Krein *et al.* 2012). One effective biological assay for water contamination is the yeast estrogen screen (YES), which is a tool for measuring chemicals with estrogenic activity in water samples and provides a measure of the overall effect of the sum of them (Routledge & Sumpter 1996; Smith *et al.* 2015). The right combination of yeast screen and analytical methods has the ability to monitor both specific and unknown pollutants (Krein *et al.* 2012).

There are only a small number of studies available in the international literature regarding the effectiveness of riverbank filtration (RBF) in removing chemicals with estrogenic activity. During RBF, the water from the river passes through nearby soil and is drawn up through wells. The process may directly yield drinkable water or need further purification. Based on the study of Hoppe-Jones *et al.* (2010), RBF systems in different geographic areas of the United States are able to act as a reliable barrier for trace organic chemicals, including BPA, if a sufficient retention time is maintained. However, EDCs, including herbicides and one pharmaceutical, were detected in all purification steps at three bank filtration sites in Nebraska, United States (Heberer *et al.* 2001). Investigations are needed to answer questions about EDCs and their behavior during the bank filtration process.

In this study, we intended to reveal how effective RBF and the total drinking water treatment process are in the removal of estrogenic compounds. We investigated the wastewater effluents of wastewater treatment plants (WWTPs) affecting the quality of river water, riverbank infiltrated water and drinking water purification steps sampled in different seasons using HPLC-MS and a yeast assay.

HPLC-MS quantified the amount of three widely known estrogenic EDCs (BPA, E2 and EE2), and the yeast assay evaluated the total estrogenic activity of the estrogenic EDCs present in the samples.

MATERIALS AND METHODS

Sampling sites and design of sampling

The waters were investigated at two RBF sites (DWTP 1 and DWTP 2), which are located in alluvial sand and gravel aquifers having hydraulic conductivities of 15–150 m/day and thickness of exploited aquifers ranges from 1.5 to 15 m. The distance between the riverbank and production wells is >20 m and travel times are 0–20 days. Water was extracted along a riverbed using several vertical and collector wells with laterals.

At DWTP 1, well water is directed to the drinking water system after chlorination. At DWTP 2, the well water after ozonation, sand filtration and chlorination is directed to the water distribution system. The schematic illustrations of the two different drinking water systems are shown in Figure 1. Both DWTPs are situated on the same river, which is the recipient of treated communal and industrial sewages. The sampling points of DWTP 1 were sewage water effluent (treated sewage affected the quality of river water), river water, RBF raw water (well), water before chlorination, and drinking water (at waterworks and at the consumer endpoint). The sampling points of DWTP 2 were sewage water effluent (treated sewage affected the quality of river water), river water, RBF raw water (well), water after ozonation, water after sand filtration, and drinking water (at waterworks and at consumer endpoints in two locations). Comparing the two DWTPs, the aquifer system of DWTP 2 is more vulnerable to pollution coming either from the river or from shallow groundwater. This area is industrialized; a highway with very heavy traffic crosses here and there is also agricultural activity. The treated sewage waters were collected from WWTPs processing 200,000 (DWTP 1) and 80,000 (DWTP 2) m³ sewage per day and equipped with modern technologies for mechanical, biological treatment, nitrogen, phosphorous removal and final UV disinfection/chlorination.

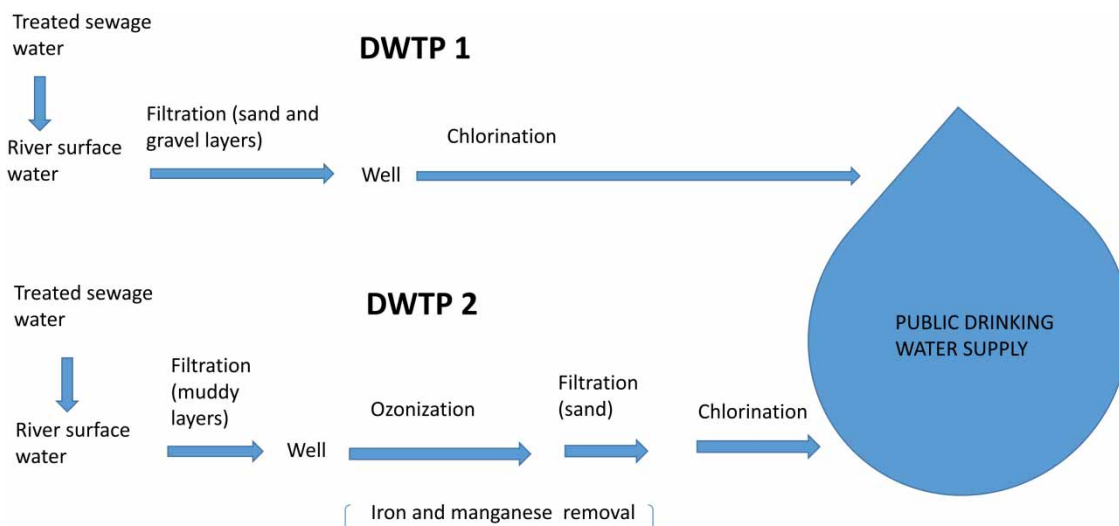


Figure 1 | Sampling sites at Hungarian drinking water treatment plants.

During the investigation period, each site was sampled twice, once in the fall of 2015 and once in the spring of 2016. The mean water levels and runoff of the raw surface water were 76 cm and 750 m³/s in the fall and 305 cm and 3,000 m³/s in the spring.

Solid phase extraction

One liter of each collected water sample was concentrated by solid phase extraction (SPE) using StrataTM X Phenomenex polymeric reversed phase (200 mg/6 mL, 8B-S100-FCH). All glassware used for YES was washed, rinsed twice with ethanol and dried at 120 °C for 2 hours. Before extraction, 10 mL methanol was added to the 1 L water sample. The suspended particles were then removed by filtration through paper filters with pore sizes of 0.2 µm (Durapore[®] membranes made with Polyvinylidene fluoride (PVDF)) to avoid SPE cartridge clogging. The SPE cartridge was activated with 8 mL methanol and later washed with 8 mL of a water:methanol solution (95:5). Next, the water sample was loaded into the SPE column with a flow rate of 6 mL/min. The cartridge was washed with 10 mL of methanol:water (1:1), followed by 10 mL of acetone:water (1:2), and then dried. Finally, the estrogenic chemicals were eluted with 10 mL of methanol and the solvent was concentrated to 500 µL using a slow nitrogen gas flow. The extract was stored at −20 °C in a 1.5-mL glass vial

with a screw cap until final analysis (Hong 2012). The mean recovery of this SPE method 87, 90 and 129%, when distilled water were spiked with 2, 1 and 0.1 ng/L E2, respectively (Hong 2012).

YES procedure

The estrogenic activity of SPE samples was evaluated using a recombinant yeast strain *Saccharomyces cerevisiae* BJ1991 according to the protocols detailed by Routledge & Sumpter (1996) with modifications as described by Hong (2012). The human estrogen receptor (hER) gene was stably integrated into the genome of yeast cells BJ1991. When an estrogen receptor agonist binds to hER, the receptor–ligand complex capable of binding to the ERE (estrogen response elements in expression plasmids) and the transcription of the reporter gene Lac-Z is initiated, β-galactosidase is synthesized. In the presence of β-galactosidase, the chromogenic substrate chlorophenol red β-d-galactopyranoside (CPRG) in the medium undergoes a color change from yellow to red. The change of absorbance can be measured at 540–580 nm (Xiao et al. 2016).

For the analysis of estrogenic activity, 10 µL aliquots of the extracted samples (cc. 2000×) were transferred to the wells of a sterilized 96-well optical flat bottom microtitre plate (Nunc, Germany), and the solvent was allowed to evaporate until it was dry. The wells were then supplied

with 175 μL of the assay medium containing yeast cells, and the covered plates were incubated at 30 °C in an incubator (PLO-EKO Aparatura) for 1 day. Next, 25 μL of CPRG (40 mg/mL) was added to each well and the plates were incubated for two more days. The color development was measured at 540 nm, and the turbidity of the yeast cell biomass was read at 620 nm (Labsystems Multiskan MS). The initial absorbance at 620 nm was adjusted to 0.1. Concentrations of the standard E2 (2.7 pg/L to 2,700 ng/L in methanol) were also analyzed in parallel as a positive control, and each plate contained negative control wells consisting of methanol alone, and blank wells that contained no organism but were treated in the same way as the other replicates in the sample. Each test substance was analyzed in duplicate and repeated three times. The relative growth was calculated to assess possible toxic effects of the sample. The mean corrected absorbance was used for subsequent statistical evaluation and the construction of a concentration-response curve (Hong 2012). The calibration of the standard curve was performed with the four-parametric logistic function (Findlay & Dillard 2007). To determine E2 estradiol equivalents (EEQ), the absorbance of the sample extracts was interpolated in the linear range of the corresponding estradiol standard curve (Hong 2012). The obtained EEQ concentration shows that the estrogenic activity of the sample is equivalent to the estrogenic activity of an equally concentrated E2 solution. The detection limit (LOD) of the yeast assay for the E2 standard was 27 pg/L, while the lowest limit of quantification (LOQ) was 0.5 ng/L EEQ. The accuracy of this method is 92–99% (confidence levels 95%), the precision is 83%, however at the lowest concentrations it is only 58% (Hong 2012).

HPLC-MS

E2, EE2 and BPA content of the concentrated (cc. 2000 \times) water samples was determined by HPLC-MS. Samples

were extracted using the same protocol as defined by YES procedure, but in order to enhance sensitivity they were derivatized with dansyl chloride prior to injection. The derivatization procedure and the analysis was performed as described in our previous works (Avar *et al.* 2016a, 2016b) with small modifications: Fifty μL of each derivatized sample was injected three times. The initial composition of the gradient was 50% solvent B (0.01% v/v formic acid in acetonitrile) and it was kept constant for 5 minutes. The percentage of eluent B was increased to 99 in 3 minutes. B was kept at 99% for 6.9 minutes, and the column was equilibrated for 15 minutes. Capillary temperature was set to 300 °C while the probe heater temperature was 450 °C. RF of the S-lenses was set to 100. Sheath and auxiliary gas flow rates were set to 80 and 20 arbitrary units, respectively. One arbitrary unit of sweep gas was applied. The energy in the high-energy collisional-induced dissociation (HCD) cell was set to 50% by E2 and EE2 and 35% by BPA.

Detection limits were BPA: LOQ 0.05 ng/L, LOD 0.01 ng/L; E2 LOQ: 0.1 ng/L, LOD: 0.03 ng/L; EE2 LOQ: 0.1 ng/L, LOD: 0.03 ng/L. Retention times and detected ions of the followed transitions (in m/z) are summarized in Table 1. Chromatographic separation of BPA, E2 and EE2 using 250 pg derivatized standard of each analyte are shown in Figure 2.

RESULTS

Fifteen samples were taken from DWTP 1, 16 samples from DWTP 2, four samples from sewage treatment plants and two samples were taken per sampling points. In total, 35 samples were analyzed. For all of the samples of the YES, clear concentration response curves were constructed, which allow for calculating the estrogenic activity in eight samples. In 20 samples, the estrogenic activity was below the LOD, and for another seven out of 35 sample extracts,

Table 1 | Retention times and detected ions of the followed transitions (in m/z)

Compound	Retention time	Theoretical m/z (parent ion)	m/z (parent ion)	m/z (daughter ion)
BPA	11.20 \pm 0.1 min	695.22440	695.22 \pm 2.0 Da	171.10 \pm 0.01 Da
E2	10.05 \pm 0.1 min	506.23596	506.24 \pm 2.0 Da	171.10 \pm 0.01 Da
EE2	10.18 \pm 0.1 min	530.23596	530.24 \pm 2.0 Da	171.10 \pm 0.01 Da

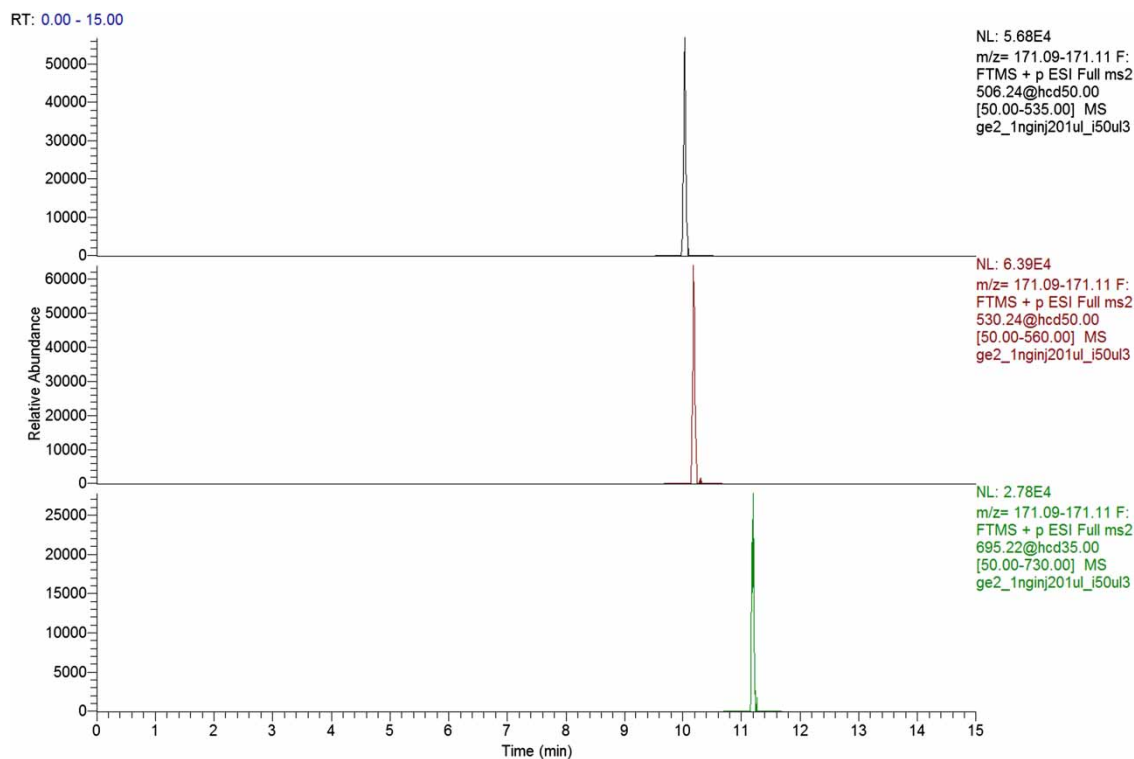


Figure 2 | Chromatographic separation of BPA (upper chromatogram), E2 (chromatogram in the middle) and EE2 (bottom chromatogram); 250 pg derivatized standard of each analyte on column.

a low signal was observed, which could not be quantified. The relative growth of the yeast was between 0.9 and 1.1, therefore none of the samples showed toxicity on yeast cells. Detailed results of the yeast assay and the HPLC-MS measurements are shown in [Tables 2 and 3](#) and [Figures 3 and 4](#).

Results of DWTP 1

In the case of DWTP 1, the treated sewage water in both the fall and spring (6.8 and 25.7 ng/L EEQ), while river water only in spring, showed estrogen activity (1.2 ng/L EEQ) according to the yeast screen.

The treated sewage water included 49.1 and 23.0 ng/L BPA (fall and spring, respectively) and 0.8 ng/L EE2 (fall). The river water contained 4.1 and 12.1 ng/L BPA (fall and spring, respectively) and 0.4 ng/L E2 (both in the fall and spring). Based on our observation, at all treatment stages (from RBF water to consumers) BPA and E2 were present at low concentrations (0.3–6.5 ng/L BPA and 0.1–1.8 ng/L E2).

Results of DWTP 2

In the case of DWTP 2, the treated sewage water in both the fall and spring had estrogen activity less than LOQ, while river water only in spring (0.5–9.2 ng/L EEQ), showed estrogen activity according to the yeast screen. Interestingly, spring samples (corresponding to higher river water level) from all treatment stages and drinking water all the time at consumer endpoint b showed low estrogenic activity below the LOQ or the range was 1.4–2.1 ng/L EEQ.

BPA concentrations in treated sewage were 67.4 and 56.6 ng/L (fall and spring, respectively) and E2 3.1 ng/L (fall). The river water contained 15.9 and 5.4 ng/L BPA and 1.1 and 1.5 ng/L E2 (in fall and spring, respectively). At all treatment stages (from RBF water to consumers), BPA and E2 were present at low concentrations (<0.05–7.1 ng/L BPA and 0.3–1.5 ng/L E2), except raw well water, where the BPA concentration was striking in the spring (25 ng/L) and tap water at consumer b in the fall with 30.7 ng/L BPA content. Chemical results did not show clear correlation with YES assay.

Table 2 | Characterization of the total estrogenic burden at DWTP 1. <LOD: below the limit of detection, LOD_{EEQ}: 0.027 ng/L, LOD_{BPA}: 0.01 ng/L, LOD_{E2}: 0.03 ng/L, LOD_{EE2}: 0.03 ng/L

Sampling date	Sampling place	EEQ ng/L	BPA ng/L	E2 ng/L	EE2 ng/L
2015 – Spring, preliminary investigations on raw surface river water					
08.03.2015	Raw river water	1.2 ± 0.8	22.6 ± 0.678	0.8 ± 0.08	<LOD
2015 – Fall					
19.11.2015	Sewage water effluent	6.8 ± 6.8	49.1 ± 1.473	<LOD	0.8 ± 0.024
25.11.2015	Raw, river water	<0.5	4.1 ± 1.927	0.4 ± 0.048	<LOD
25.11.2015	East, RBF water	<LOD	3.5 ± 0.525	<0.1	<LOD
25.11.2015	East, treated (final) water	<LOD	3.1 ± 0.837	0.1 ± 0.03	<LOD
25.11.2015	West, RBF water	<LOD	6.5 ± 0.52	0.2 ± 0.018	<LOD
25.11.2015	West, before chlorination	<LOD	4.8 ± 0.192	0.2 ± 0.018	<LOD
25.11.2015	West, treated (final) water	<LOD	0.3 ± 0.048	0.1 ± 0.006	<LOD
20.11.2015	Drinking water at consumer endpoint	<LOD	2.9 ± 0.116	0.4 ± 0.036	<LOD
2016 – Spring					
07.03.2016	Sewage water effluent	25.7 ± 21.9	23.0 ± 0.69	<LOD	<LOD
01.03.2016	Raw, river water	<LOD	12.1 ± 0.726	0.4 ± 0.12	<LOD
01.03.2016	East, RBF water	<LOD	1.5 ± 0.165	0.4 ± 0.052	<LOD
01.03.2016	East, treated (final) water	<LOD	0.9 ± 0.135	1.1 ± 0.022	<LOD
01.03.2016	West, RBF water	<LOD	2.3 ± 0.138	1.8 ± 0.018	<LOD
01.03.2016	West, after chlorination	<LOD	0.9 ± 0.108	0.2 ± 0.042	<LOD
01.03.2016	West, treated (final) water	<LOD	1.3 ± 0.26	0.3 ± 0.078	<LOD
03.03.2016	Drinking water at consumer endpoint	<LOD	1.8 ± 0.09	0.9 ± 0.036	<LOD

DISCUSSION

In our study, we combined biological screening with targeted HPLC-MS measurements. The YES has been deemed as a rapid and sensitive means of assessing estrogenic activity without identifying specific chemical components of the extracts of the environmental samples while providing information on the total effect of pollutants acting together in the mixtures (ISO 2017). We have detected estrogenic activity in raw river water and sewage water effluents at both DWTPs and estrogenic activity was under the detectable amount both in RBF waters and in later treatment phases at DWTP 1 in fall 2015 and spring 2016 and at DWTP 2 in fall 2015. Interestingly, YES achieved in the spring of 2016 in DWTP 2 showed weak positivity during the entire treatment, from RBF well water until the treated (final) water. From the point of view of water treatment efficiency, the RBF, ozonation and chlorination combination did not decrease the estrogenic activity.

The levels of estrogenic activity in sewage water effluent are highly variable in Europe and dependent on the intake and treatment processes (Tiedeken *et al.* 2017). The estrogenic activity in the surface water in Hungary is a similar magnitude as detected in Switzerland (0.3–7 ng/L), Catalonia (mainly <0.5 ng/L), but lower than reported from Luxemburg (up to 20.77 ng/L) and from the UK (0.04–23.21 ng/L) (Céspedes *et al.* 2005; Vermeirssen *et al.* 2005; Jobling *et al.* 2009; Krein *et al.* 2012). Our measured estrogenic activity is lower, which may contribute to reproductive disturbances of fish and aquatic life as predicted not effective concentrations for E2 are 2–8.7 ng/L and for EE2 are 0.035–0.5 ng/L (Liney *et al.* 2005; Adeel *et al.* 2017). The median effective concentration (EC₅₀) values for vitellogenin induction in juvenile brown trout were 3.7 ng EE2/L and 15 ng E2/L (Bjerregaard *et al.* 2008). Based on the revised drinking water directive the parametric value of 1 ng/L E2 was proposed for drinking water (EC 2018). None of the drinking water samples

Table 3 | Characterization of the total estrogenic burden at DWTP 2. <LOD: below the limit of detection, LOD_{EEQ}: 0.027 ng/L, LOD_{BPA}: 0.01 ng/L, LOD_{E2}: 0.03 ng/L, LOD_{EE2}: 0.03 ng/L

Sampling date	Sampling place	EEQ ng/L	BPA ng/L	E2 ng/L	EE2 ng/L
2015 – Spring, preliminary investigations on raw surface river water					
08.03.2015	Raw river water sampling point 1	0.5 ± 0.3	19.8 ± 1.386	1.0 ± 0.09	<LOD
08.03.2015	Raw river water sampling point 2	9.2 ± 2.0	32.4 ± 1.944	2.2 ± 0.088	<LOD
2015 – Fall					
19.11.2015	Sewage water effluent	<0.5	67.4 ± 5.392	3.1 ± 0.186	<LOD
12.11.2015	Raw, river water	<LOD	15.9 ± 0.795	1.1 ± 0.143	<LOD
12.11.2015	RBF water	<LOD	7.1 ± 0.568	0.3 ± 0.027	<LOD
12.11.2015	After ozonation	<LOD	5.5 ± 0.77	0.8 ± 0.088	<LOD
12.11.2015	After sand filtration	<LOD	2.4 ± 0.096	0.6 ± 0.12	<LOD
12.11.2015	Treated (final) water	<LOD	3.0 ± 0.06	0.6 ± 0.12	<LOD
20.11.2015	Drinking water at consumer endpoint a	<LOD	<0.05	<LOD	<LOD
06.11.2015	Drinking water at consumer endpoint b	<0.5	30.7 ± 0.921	<LOD	<LOD
2016 – Spring					
07.03.2016	Sewage water effluent	<0.5	56.6 ± 1.698	<LOD	<LOD
02.03.2016	Raw, river water	2.1 ± 2.1	5.4 ± 0.27	1.5 ± 0.165	<LOD
02.03.2016	RBF water	<0.5	25.0 ± 2.25	1.4 ± 0.07	<LOD
02.03.2016	After ozonation	1.4 ± 1.4	0.3 ± 0.036	1.2 ± 0.096	<LOD
02.03.2016	After sand filtration	<0.5	2.7 ± 0.189	0.8 ± 0.136	<LOD
02.03.2016	Treated (final) water	<0.5	1.0 ± 0.14	0.7 ± 0.042	<LOD
08.03.2016	Drinking water at consumer endpoint a	<LOD	1.2 ± 0.12	0.6 ± 0.09	<LOD
10.03.2016	Drinking water at consumer endpoint b	1.6 ± 1.6	0.5 ± 0.1	2.0 ± 0.04	<LOD

exceeded this limit in our preliminary study as measurements were conducted on 2000× water concentrates. Research on the reduction of estrogenic activity by DWTP-s is scarce in Europe. A paper from France showed no estrogenic activity after drinking water processes by luciferase reporter gene assays using PC-DR-LUC and MELN cells (Jugan *et al.* 2009). Comparisons between studies are very difficult, as different laboratories use different protocols for sample concentration and the YES and communicate the results in different ways.

In our HPLC-MS measurements, we found BPA in all water types. Sewage effluents, river water and water from consumer endpoint 'b' contained higher concentrations, but there was no apparent trend. BPA measurements showed concentrations of 0.5–410 ng/L in the surface water in Germany and in other German surveillance, BPA was found in concentrations ranging from 0.3 to 2 ng/L in drinking water samples (Kuch & Ballschmiter 2001;

Fromme *et al.* 2002). We found similar BPA concentrations of 4.1–32.4 ng/L in surface water in Hungary; however, the range of BPA concentration in drinking water is higher in this study (BPA 0.3–30.7 ng/L). BPA is common in epoxy resins and plastics (PVC). Epoxy resins are used in protective linings to reduce leaks, and damaged water pipes, instead of being completely replaced, can be relined with epoxy-based coatings (Cooper *et al.* 2012). Pipes lined with the older LSE (LSE-SYSTEM AG) technology using LSE-001 NA epoxy coating material leach more BPA than those with the new DonPro (Donauer & Probst GmbH & Co) technology using Tubeprotect epoxy coating material: the maxima in cold water could be 0.25 mg/L and 10 ng/L, respectively. Stagnation of water in pipes prior to sampling increases the BPA concentration in cold water (Rajasärkkä *et al.* 2016). Therefore, the striking BPA content of consumer endpoint 'b' may originate from the old and repaired water pipes. Based on the revised drinking water

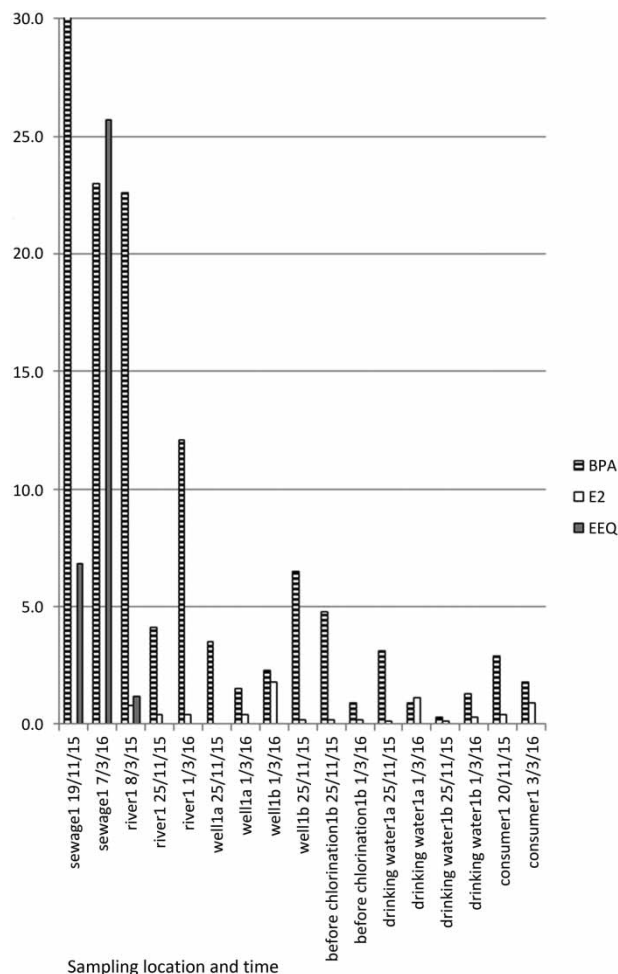


Figure 3 | Measured BPA, E2 concentrations and EEQ at DWTP 1.

directive, the parametric value of 10 ng/L BPA was proposed for drinking water (EC 2018). None of the drinking water samples exceeded this limit in our preliminary study as measurements were conducted on 2000× water concentrates. The natural estrogens (E2) and their main metabolites (E1, E3) are discharged via sewage or manure. The speed of biodegradation of these substances is often too slow (half-life up to 5 days) to allow complete removal before they reach water sources (Wenzel *et al.* 2003; Adeel *et al.* 2017). Synthetic estrogen EE2 is more persistent in the environment than natural estrogens, and their presence in water is a greater cause for environmental concern (Adeel *et al.* 2017). During HPLC-MS measurements, E2 (0–3.1 ng/L) was continuously present in all water types sampled without extremely high concentrations. EE2

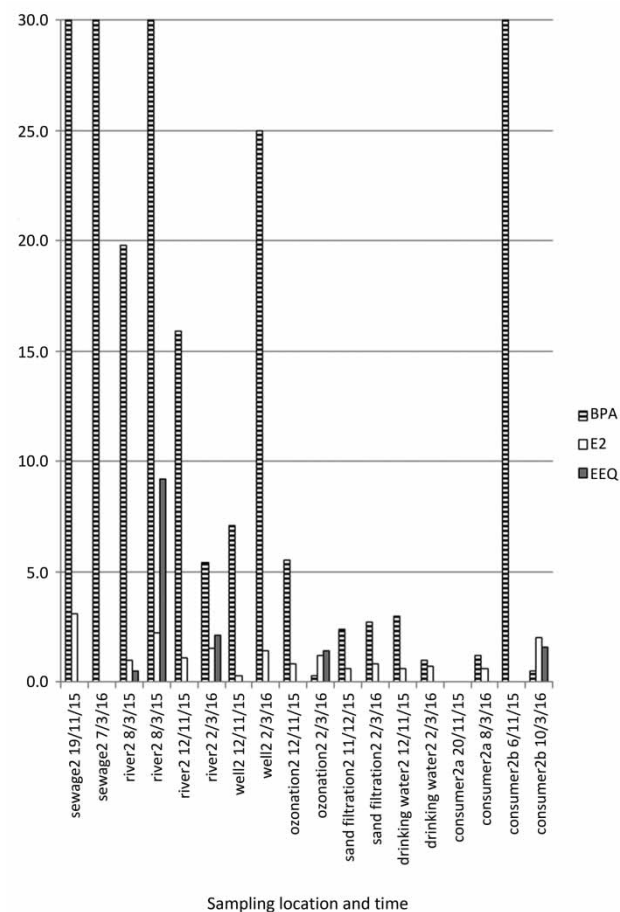


Figure 4 | Measured BPA, E2 concentrations and EEQ at DWTP 2.

(0.8 ng/L) was observed only in treated sewage water in the fall of 2015. Based on our previous studies, river water samples contained 0–5.2 ng/L E2 and 0–0.68 ng/L EE2 in Hungary, which is consistent with our present finding in surface raw river water (Avar *et al.* 2016b). From a European perspective, a survey of contamination of Lake Maggiore in Italy detected estrone (E1) at 0.4 ng/L in the raw lake water and levels of these compounds in drinking water were almost identical with those found in the raw water itself, showing the poor performance of sand filtration and chlorination combination at the local waterworks (Loos *et al.* 2007). In all river water samples in a German survey, the steroids were 0.2–5 ng/L and in drinking water were 0.1–2 ng/L (Kuch & Ballschmiter 2001). We found similar E2 concentrations to these European findings in both surface and drinking water in our study (0.4–2.2 and 0–2 ng/L of E2, respectively).

Toxicological risk assessments for EDCs are complicated by multiple routes of exposure, by nonmonotonic dose-response curves where responses both increase and decrease across the dose range or by interactions among chemicals within mixtures (Jobling *et al.* 2009). Risk assessments conducted to date do not confirm that the ng or pg concentrations of hormones and hormone metabolites detected in drinking water pose a health risk to consumers, but most of these assessments are based on comparisons of concentrations in water with therapeutic doses, which are much greater than doses that could be attained through contaminated drinking water. Research to date is inconclusive regarding the health effects of low-level estrogenic compounds in the water supply and responses to low doses should be determined (Snyder *et al.* 2009). Through the Water Framework Directive, E1, E2 and EE2 have been added to the European Union watch list of priority substances to be monitored. Furthermore, specific legislative obligations have been introduced by the European Union aimed at the phasing out of endocrine disruptors in industrial chemicals, cosmetics, plant protection products and biocides (Tiedeken *et al.* 2017; Updates of Endocrine Disruptors Regulations and Lists in EU 2016).

The estrogenic potential of a chemical is expressed as a relative potency to the reference compound 17 β -estradiol (E2). If the potency of 17 β -estradiol (E2) is 100%, the relative potency of ethinyl-estradiol (EE2) is 88.8% and bisphenol A (BPA) is 0.005%, which were measured in this study (Coldham *et al.* 1997). In general, it is expected that the measured activity in the YES, which includes all potential estrogenic chemicals, is higher than the calculated activity based on HPLC-MS measurements. Jobling *et al.* (2009) demonstrated that the chemical analysis and the YES are not comparable and the estrogenic activity (EEQ) of the water samples did not correlate well with the concentrations of individual steroidal estrogens measured, which is supported by our study. This lack of correlation could be due to the presence of anti-estrogenic compounds in the samples, which would reduce the response seen in the yeast assay. The signal obtained by YES is more relevant from the water quality perspective as interactions between chemicals are detected and therefore could have a higher predictive value when possible effects need to be measured (Jobling *et al.* 2009).

CONCLUSIONS

Our study confirms that estrogenic chemicals are present in sewage water effluents and raw surface river water of DWTPs. Very low estrogen activity and pg/L concentrations of BPA and E2 have been detected during drinking water processing and occasionally in drinking water. RBF and applied water treatment procedures do not seem to be suitable for the total removal of estrogenic compounds. Local contaminations can play a role in increasing the BPA content of the drinking water at the consumer endpoint. Further extensive studies are necessary at drinking water treatment plants using surface river water, which combine biological assays with the measurement of carefully selected chemical compounds adapted to local features. Our data provide limited information on BPA and E2 concentrations and estrogenic compounds in drinking water. The database should be enhanced to offer a wider picture, and samples should be analyzed at the consumer endpoint.

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